A MOLECULAR GENETIC AND MORPHOLOGICAL APPRAISAL OF A DISTINCTIVE FORM OF THE SANDY INLAND MOUSE, *PSEUDOMYS HERMANNSBURGENSIS*

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SUMMARY

The molecular genetic techniques of allozyme electrophoresis and mitochondrial DNA sequencing were used to assess the genetic and taxonomic affinities of a "gracile" form of the Sandy Inland Mouse *Pseudomys hermannsburgensis* from the Pilbara region of Western Australia. Measurement of four external variables plus 26 cranial and dentary variables were also made to assess the nature of any morphological differences between the distinctive animals and typical *P. hermannsburgensis*. Individuals of each morphotype plus other reference specimens and species were genotyped at 53 allozyme loci and sequenced for a 307 base-pair portion of the mitochondrial *cytb* gene. While the most obvious differences between the two morphotypes involve external characters, there is some suggestion that the relative shape of the skull may also be partly diagnostic. Despite these morphological differences, the genetic data reveal no evidence that the two forms are genetically distinct from one another and thus demonstrate they are highly likely to be conspecific.

INTRODUCTION

Recent survey work in the Pilbara region of Western Australia has revealed several adult specimens of the Sandy Inland Mouse, *Pseudomys hermannsburgensis*, whose appearance is distinctive from other specimens in the region. These distinctive animals appear smaller and more gracile, comparative in size to a large *P. delicatulus*, when compared to animals displaying the typical appearance of an adult *P. hermannsburgensis*. Importantly, these gracile animals are sympatric with typical *P. hermannsburgensis*, thus ruling out the possibility that any morphological differences have resulted from the genetic

isolation of a regional subpopulation of *P. hermannsburgensis.*

Two hypotheses could account for these unusual animals, namely (1) they represent a previously-undocumented morphological variant within the species, or (2) they represent another, presumably new, species of *Pseudomys.* Given that several species described in the past 20 years were formerly included in *P.* "hermannsburgensis" (Breed 1995), any variant form of the Sandy Inland Mouse is clearly a prime candidate for consideration as a new species.

Distinguishing between these alternative explanations is best achieved using molecular genetic data to provide an independent assessment of the genetic affinities of the two forms. Of the many techniques available, allozyme electrophoresis and mtDNA sequencing are the most suitable and cost-effective molecular techniques (Avise 1994). The former can determine whether the two forms are members of the same biological species, whilst the latter can resolve the evolutionary affinities of the variant form amongst other related species of *Pseudomys* (to ensure that any "new" taxon does not turn out to be a variant of an already-described species).

Previous field workers have also recognised two morphotypes of *P. hermannsburgensis* in the field, (*pers. comm.* Norm Mckenzie, Ken Aplin, and Roy Teale), although unfortunately no aberrant specimens had tissues taken for molecular genetic analysis. However, during a survey in November 1998 at Brockman Mine in the Pilbara (22°18"S, 117 °17'E), several animals representing both the standard and gracile morphotypes were vouchered at the Western Australian Museum for both the preserved specimen and their frozen tissues. The chance to obtain molecular and morphological data from sympatric representatives of both forms provides the ideal opportunity to determine whether the gracile form is indeed a new species, as suggested by its morphological distinctiveness, or simply an unusual variant of the Sandy Inland Mouse. The purpose of this paper is to determine the taxonomic affinities of the two morphological forms using molecular genetic and morphological techniques.

METHODS

Specimens were trapped in the field using Elliott Traps or Pit Trap/fences. Animals were euthanased, numbered, and livers were immediately removed into liquid nitrogen. The animal was then preserved in 4% formalin before being washed and stored in 70% ethanol. Skulls were removed and cleaned for morphological comparison.

Morphological measurements

Given that, by definition, "unusual" morphotypic forms are

only rarely encountered in the field and even less commonly taken as vouchers, the small sample size (n=5) available for the gracile form (hereinafter referred to as *Pseudomys* sp. for convenience) precludes any comprehensive statistical analyses of the morphological data. This limitation of small sample size is further compounded by the fact that gender and adult age class differences can never be properly explored in any rare taxon (ie. *Pseudomys* sp.). As such, the best that can be achieved herein is a qualitative assessment of the morphological differences between the two forms, in combination with a basic Analysis of Variance for each individual character. In regard to the latter, it must be remembered that, due to the small sample sizes involved, the chance of a "type II" statistical error (i.e. falsely accepting the null hypothesis that the two forms do not differ significantly for a given character) remains unavoidably high in any univariate statistical test.

Only adult animals were included in this study. Animals were considered adult if they were reproductively mature and if skull sutures were fused. Two age classes were observed, based on dental differences; class 3 = mature adult with worn teeth and class 2 = adult with slight wear on teeth. Although all five *Pseudomys* sp. available were males, both male (n=4-5) and female (n=3) *Pseudomys hermannsburgensis* were included in the morphological analyses to provide a more complete picture of morphological diversity in the "parent" taxon. The details of all animals subjected to morphological analysis are presented in Table 1.

External and cranial measurements of all specimens were made using digital callipers. Four external characters were measured, namely: HV, head vent length; TV, tail-vent length; PESL, pes length; and EARL, ear length (Table 2a). The following cranial measures were recorded (Table 2b), as outlined in Cooper *et al.* (2003): GL, greatest length; BD, braincase depth; IPW, interparietal width; BW, braincase width; ZW, zygomatic width; IW, interorbital width; NL, nasal length; NW, nasal width; BUW, bulla width; BL, braincase length; PL, palate length; M^1L, upper molar 1 length; M^3L, upper molar 3 length; M^1M^3, upper molar 1 to molar 3 length; APF, anterior palatal foramen; CIL, condyle to incisor length; and M_1M_3, lower molar 1 to molar 3 length. In addition, a series of extra cranial measurements, not used by Cooper *et al.* (2003), were taken: BUL, bulla length; WOB, width outside bullae; WIB, width between bullae; M^2L, upper molar 2 length; MTD, mesopterygoid distance; PTD, pterygoid distance; PTW, width of pterygoid at tip.

To qualitatively assess the extent to which the two forms were diagnosable using the morphological data, a separate Principal

Components Analysis (PCA) was performed on each of the external and cranial datasets. The statistical significance of any differences between the two forms was assessed for each variable by Analysis of Variance (ANOVA). Both procedures were carried out using the computer program *STATISTICA*.

Allozyme analysis

Frozen livers were available in the tissue collections of the Western Australian and South Australian Museums. Four specimens of *Pseudomys* sp. from two locations were available for molecular analysis. These were compared to 13 typical *Pseudomys hermannsburgensis* taken from six locations, including two sites where both forms co-exist. Single specimens of *P. bolami* and *P. chapmani*, two species previously considered conspecific with *P. hermannsburgensis*, were also included. The details of all specimens screened are contained in Table 1.

Allozyme electrophoresis was undertaken on cellulose acetate gels according to the principles and procedures detailed in Richardson *et al.* (1986). A total of 53 loci displayed sufficient activity and resolution after electrophoresis to allow allozymic interpretation. Details of the locus abbreviations are presented in Georges and Adams (1992) whilst the nomenclature used to refer to loci and allozymes follows Adams *et al.* (1987). The statistical significance of any differences in allozyme frequency between the two morphotypes was assessed using the computer program GENEPOP (Raymond and Rousset 1995).

mtDNA sequencing

Two individuals of the variant morphotype plus one typical *P. hermannsburgensis* were sequenced for a 307 base-pair portion of the cytochrome-b (*cytb*) mitochondrial gene, according to standard protocols (Hillis *et al.* 1996). These sequences were compared to an existing *cytb* database previously generated in our laboratory and comprising *P. hermannsburgensis* plus its near relatives. The data were analysed both by eye and by constructing a neighbour-joining tree from the Jukes-Cantor genetic distance matrix, using the computer program MEGA (Kumar *et al.* 1993). Table 1 provides details of which animals were included in the mtDNA study.

RESULTS

External morphology

From initial comparison of the bodies of the two morphs, the *Pseudomys* sp. individuals, though fully reproductively mature with descended testes and epididymis, were obviously smaller than fully mature males of *P. hermannsburgensis*, even in sympatry (Brockman Mine). There, a mature male *P.*

Table 1. Details of all specimens used in this study. Code for Accession Numbers: WAMM = Western Australian Museum, SAMM = South Australian Museum, AMSM = Australian Museum; ABTC = Australian Biological Tissues Collection. Code for Data column: A = allozymes, S = skull measurements, E = external measurements, D = mtDNA sequence.

Accession Number	Taxon	Data	Locality	Latitude	Longitude
WAMM52452	*Pseudomys* sp?	A,S,E	Brockman Mine, WA	22°18'38"	117°19'19"
WAMM52437	*Pseudomys* sp?	A,S,E,D	Brockman Mine, WA	22°17'31"	117°18'03"
WAMM44138	*Pseudomys* sp?	A,E,D	Karratha, WA	20°40'16"	117°04'52"
WAMM41305	*Pseudomys* sp?	A,S,E	Karratha, WA	20°40'16"	117°04'52"
WAMM52371	*Pseudomys* sp?	S,E	Cape Preston, WA	21°03'57"	116°08'57"
WAMM52425	*P. hermannsburgensis*	A,S,E,D	Brockman Mine, WA	22°24'51"	117°21'18"
WAMM52411	*P. hermannsburgensis*	A,S,E	Brockman Mine, WA	22°17'31"	117°17'56"
WAMM52410	*P. hermannsburgensis*	A	Brockman Mine, WA	22°18'39"	117°18'40"
WAMM44139	*P. hermannsburgensis*	A	Karratha, WA	20°40'16"	117°04'52"
WAMM41304	*P. hermannsburgensis*	A,S	Karratha, WA	20°37'00"	117°10'59"
WAMM41801	*P. hermannsburgensis*	A,S	Barlee, WA	23°24'41"	115°53'39"
WAMM41813	*P. hermannsburgensis*	A,S,E	Barlee, WA	23°24'41"	115°53'39"
WAMM41833	*P. hermannsburgensis*	A,S,E	Barlee, WA	23°04'46"	115°47'27"
WAMM41837	*P. hermannsburgensis*	A	Barlee, WA	23°06'46"	116°00'28"
WAMM43170	*P. hermannsburgensis*	A,E	Nifty Mine, WA	21°40'00"	121°35'00"
WAMM48513	*P. hermannsburgensis*	A,S,E	Newman, WA	23°20'00"	119°34'00"
WAMM48514	*P. hermannsburgensis*	A,S,E	Newman, WA	23°20'00"	119°34'00"
SAMM18754	*P. hermannsburgensis*	A	Wallatinna, SA	27°25'00"	133°24'00"
SAMM14004	*P. hermannsburgensis*	D	Coongie Lakes, SA	27°08'37"	140°05'23"
AMSM25377	*P. hermannsburgensis*	D	Gerara Station, NSW	29°12'00"	146°15'00"
AMSM25376	*P. hermannsburgensis*	D	Gerara Station, NSW	29°12'00"	146°15'00"
ABTC28759	*P. hermannsburgensis*	D	Tanami Desert, NT	19°16'00"	132°40'00"

SAMM17273	*P. bolami*	A	Andrew Dams, SA	33°35'00"	139°55'00"
ABTC67582	*P. bolami*	D	Brachina Gorge, SA	31°20'00"	138°32'00"
ABTC13341	*P. bolami*	D	Balcanoona Station, SA	30°36'35"	139°26'37"
WAMM47645	*P. chapmani*	A	Newman, WA	23°29'00"	120°12'00"
WAMM34297	*P. chapmani*	D	Woodstock Station, WA	21°41'45"	119°03'15"
WAMM29449	*P. chapmani*	D	Woodstock Station, WA	21°35'15"	119°05'05"
SAMM18804	*P. australis*	D	Three Mile Well, SA	29°43'47"	137°22'33"

hermannsburgensis collected from site NA52 on 22 November 1998, weighed 11g, whereas a mature male *Pseudomys* sp. collected at the same site on the previous day weighed 8.6g (20% lighter). The *Pseudomys* sp. was physically small and gracile, more like *P. delicatulus* than *P. hermannsburgensis*. The pelage of the *Pseudomys* sp. was similar to that of *P. hermannsburgensis*. Although both were sympatric in the sense of being collected at the same site, it was not possible to determine if they were truly "syntopic" i.e. occupying the same micro-habitat.

A PCA on the external variables is presented in Fig 1a). As the presence of a missing value in one of the *P. hermannsburgensis* specimens (WAMM41833; Table 2a) was causing this specimen to appear as a significant outlier (from both *Pseudomys* sp. and the other *P. hermannsburgensis* specimens), this specimen was omitted from the final analysis. The PCA qualitatively demonstrates the distinctiveness of the four specimens of *Pseudomys* sp. when compared to typical *P. hermannsburgensis*, both male and female. Interestingly, the first Principal Component is heavily influenced by the variables HL and PESL, despite the most striking dichotomy in the raw data being found in the variable EARL (Table 2a). This observation is supported by ANOVA on the four individual characters, in which only EARL showed a statistically-significant

difference between the two morphotypes (p=>0.01 for all adult males).

Cranial morphology

From an initial observation of skulls, the cranium in *Pseudomys* sp. appears to be more rounded, the upper molars narrower, the molar row shorter, the bullae less inflated though wider, the anterior palatal foramen reaches

Table 2a. External measures for the four *Pseudomys* sp. and seven *P. hermannsburgensis* examined. Code for sex: 1 = male, 2 = female. Code for age class: 2 = adult, slight wear on teeth, 3 = mature adult, worn teeth. A dash (-) indicates a missing value.

Animal	Form	Sex	Age	HV	TV	PESL	EARL
M44138	sp	1	2	58.4	73.5	14.9	9.1
M41305	sp	1	2	56.4	69.9	16.2	8.2
M52437	sp	1	2	56.7	71.8	16.5	9.0
M52452	sp	1	2	55.9	74.1	15.7	7.6
M43170	Ph	1	2	48.0	79.0	16.7	11.7
M52411	Ph	1	2	64.0	90.0	17.4	10.2
M41833	Ph	1	3	65.0	-	14.8	10.2
M48514	Ph	1	3	63.5	79.0	16.0	10.6
M48513	Ph	2	3	63.0	80.0	16.5	12.3
M41813	Ph	2	3	61.6	72.5	16.5	12.5
M52425	Ph	2	3	62.0	77.7	16.7	10.9

Table 2b. Skull measures for the four *Pseudomys* sp. and eight *P. hermannsburgensis* examined. Codes as for Table 2a.

Animal	Form	Sex	Age	GL	BD	IPW	BW	ZW	IW	NL	NW	BUL	BUW	WOB
M41305	sp	1	2	20.67	7.37	8.03	10.32	10.64	3.22	7.01	2.46	5.21	4.85	10.08
M52437	sp	1	2	21.68	7.04	6.99	10.01	10.60	3.14	7.20	2.37	5.04	4.70	9.92
M52452	sp	1	2	19.79	6.53	6.93	-	10.12	3.41	6.89	2.54	4.86	3.70	9.77
M52371	sp	1	2	21.71	7.62	6.50	10.54	11.14	3.51	6.99	2.56	5.21	4.70	10.46
M41304	Ph	1	3	22.62	6.91	7.60	10.50	11.17	3.51	8.48	2.64	5.64	5.07	10.71
M52411	Ph	1	2	21.86	7.30	7.05	10.25	10.81	3.58	7.26	2.52	5.38	4.78	10.07
M41801	Ph	1	2	19.83	7.05	7.63	10.31	11.07	3.36	6.72	2.62	4.95	4.56	9.85
M41833	Ph	1	3	22.06	6.67	7.56	10.07	10.91	3.55	7.32	2.60	5.12	4.70	10.21
M48514	Ph	1	3	21.57	7.38	8.31	10.46	11.51	3.36	7.95	2.41	5.58	5.28	10.75
M48513	Ph	2	3	-	7.21	8.25	10.52	11.21	3.52	7.71	2.63	5.33	4.98	10.26
M41813	Ph	2	3	22.84	6.59	7.64	10.28	10.79	3.50	8.07	2.65	5.49	4.67	10.81
M52425	Ph	2	3	22.41	6.98	7.39	10.06	10.65	3.41	7.91	2.47	5.26	4.96	10.29

the anterior end of upper molar 1 (as opposed to not reaching the start of the molar row) and the pterygoids appear to be more flared and wider at the tips than that of an average *P. hermannsburgensis.*

A PCA on the skull variables (Fig 1b) indicates that while two of the four *Pseudomys* sp. have moderately distinctive scores at Principal Component 1, the other two specimens lie squarely within the *P. hermannsburgensis* cluster. Thus there is no strong indication that the two morphotypes show any qualitative differences in skull characteristics above and beyond the variance displayed within each morphotype. This conclusion is backed up by ANOVA on individual characters, which did not reveal any statistically-significant differences between the two forms. Clearly a much larger sample size is required for *Pseudomys* sp. before a definitive answer on comparative skull morphology can be given.

Allozyme analysis

Thirty-five of the 52 loci used in this study were variable, displaying between two and four alleles. The remaining seventeen loci (*Ap-3, Ca-1, Est, G6pd, Gdh, Gldh, Glo, Got-1, Gpx, Hb, Idh-2, Lap, Mdh-2, Ndpk, Pgam, Pgk,* and *Pk-2*) were monomorphic. Table 3 presents the allozyme profiles for all 19 *Pseudomys* at the 35 variable loci.

The two morphotypic forms of the Sandy Inland Mouse share alleles at all loci. This contrasts markedly when either is compared with the other two species examined. Thus *P. bolami,* a species closely-related to *P. hermanns-burgensis* and until recently considered to be conspecific (Kitchener *et al.* 1984), displayed

WIB	BL	PL	M1L	M2L	M3L	M1W	M1M3U	M1M1	MTD	PTD	PTW	APF	CIL	M1M3L
1.35	17.44	11.10	1.72	1.05	0.78	1.07	3.53	4.50	1.01	0.99	0.54	3.90	12.46	3.19
1.14	16.68	10.20	1.55	0.50	0.97	1.06	3.16	4.42	0.97	0.80	0.45	3.29	12.95	2.93
1.21	17.03	10.18	1.84	0.99	0.79	1.18	3.37	4.42	0.95	-	0.75	3.74	11.80	2.80
1.75	17.95	11.22	1.76	0.98	0.53	1.07	3.48	4.53	0.95	1.30	0.58	3.74	11.98	3.20
1.27	18.74	11.63	1.84	1.03	0.77	1.11	3.44	4.70	1.22	0.96	0.47	3.42	13.45	3.15
1.12	18.07	11.12	1.77	1.14	0.73	1.16	3.43	4.56	0.74	1.06	0.51	3.73	12.97	3.33
1.24	16.24	10.15	1.65	1.12	0.70	1.04	3.41	4.27	0.90	0.96	0.55	4.08	10.90	3.15
1.33	18.18	11.08	1.91	1.01	0.67	1.13	3.42	4.70	1.06	1.32	0.43	3.19	13.05	3.14
1.44	17.76	11.02	1.72	0.98	0.71	1.11	3.41	4.82	1.01	1.25	0.36	3.77	12.93	3.06
1.24	17.35	11.12	1.82	1.15	0.59	1.15	4.77	4.78	0.92	1.04	0.60	3.82	12.81	3.31
1.26	10.59	11.65	1.78	1.07	0.82	1.12	3.49	4.58	1.08	1.12	0.48	3.93	13.83	3.33
1.30	18.32	10.95	1.83	0.97	0.76	1.13	3.44	4.66	1.14	1.07	0.38	4.05	13.38	3.37

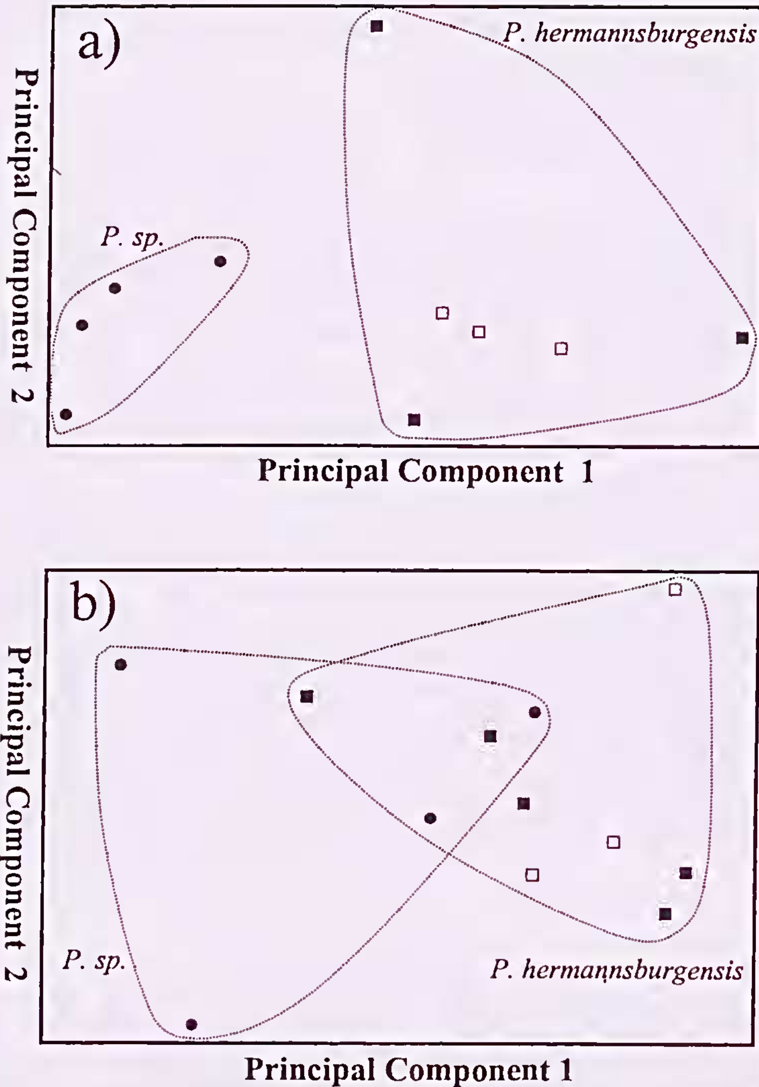

Figure 1. Principal Component Analysis of a) external and b) skull measures. Each animal has been plotted for their individual scores at the first two principal components extracted. Legend for specimens: closed circles = *P.* sp., closed squares = male *P. hermannsburgensis*; open squares = female *P. hermannsburgensis*. Specimen WAMM41833 was excluded from analysis a) due to the distortion caused by a missing value (see text for details).

fixed differences at 18% of loci (data in Table 3), and *P. chapmani* (once also included in *P. hermannsburgensis*; see Kitchener 1980) differs from both *P. hermannsburgensis* and *P. bolami* at 40-42% of loci examined. All species of *Pseudomys* examined to

Table 3. Allozyme profiles at 35 variable loci for the 19 *Pseudomys* examined. Identified by museum number and abbreviated taxon.

	Psp				*Pherm*													*Pbol*	*Paust*
Locus	52452	52437	44138	41305	52425	52411	52410	44139	41304	41801	41813	41833	41837	43170	48513	48514	18754	17273	47645
Acp	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	ab
Acyc	b	b	b	b	bc	bc	b	b	b	b	b	b	b	bc	b	b	b	bc	a
Ada	a	a	b	ab	a	a	a	ab	a	ab	a	a	a	a	ab	ab	a	a	b
Adh-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	c
Adh-2	ab	ab	b	b	b	b	b	b	b	b	b	b	b	b	b	b	ab	b	b
Ak-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ak-2	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	ab	c
Alb	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	b	a
Ap-1	b	b	b	b	b	b	b	ab	b	b	b	b	b	b	b	b	b	b	b
Ap-2	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a	c
Ca-2	b	b	ab	b	b	b	b	b	b	b	b	b	ab	b	b	b	b	b	c
Dia	b	b	b	b	b	ab	b	b	b	b	b	b	b	ab	ab	b	b	b	a
Enol	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	b	a
Fdp	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a	c
Fum	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b
Gda	b	b	b	b	b	b	b	b	b	b	b	b	b	ab	b	b	b	ab	b
Got-2	bd	bd	b	b	b	b	d	b	b	b	b	bd	b	b	bd	bd	bd	c	a
Gpi	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b
Gpt	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b
Hbdh	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b
Idh-1	a	a	a	a	a	a	a	bc	a	cd	c	ac	a	a	a	a	a	a	a
Ldh	b	b	ab	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b
Mdh-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	ab
Me	a	a	a	a	a	a	a	a	ab	a	a	a	a	a	a	a	a	a	c
Mpi	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	c	a
Np	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	ab	b
PepA	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a
PepD	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	ab
6Pgd	b	b	b	b	b	b	b	b	b	b	b	bc	b	b	b	b	b	a	d
Pgm-1	a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Pgm-2	c	c	c	c	c	c	c	c	c	c	c	ac	ad	c	c	c	c	c	bc
Pk-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b
Sod	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a
Sordh	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a
Tpi	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b

date have been diagnosable from one another using allozyme data (Baverstock *et al.* 1981).

Table 4 summarizes the allozyme frequencies for each morphotype at the 14 loci polymorphic within the Sandy Inland Mouse. The two morphotypes have

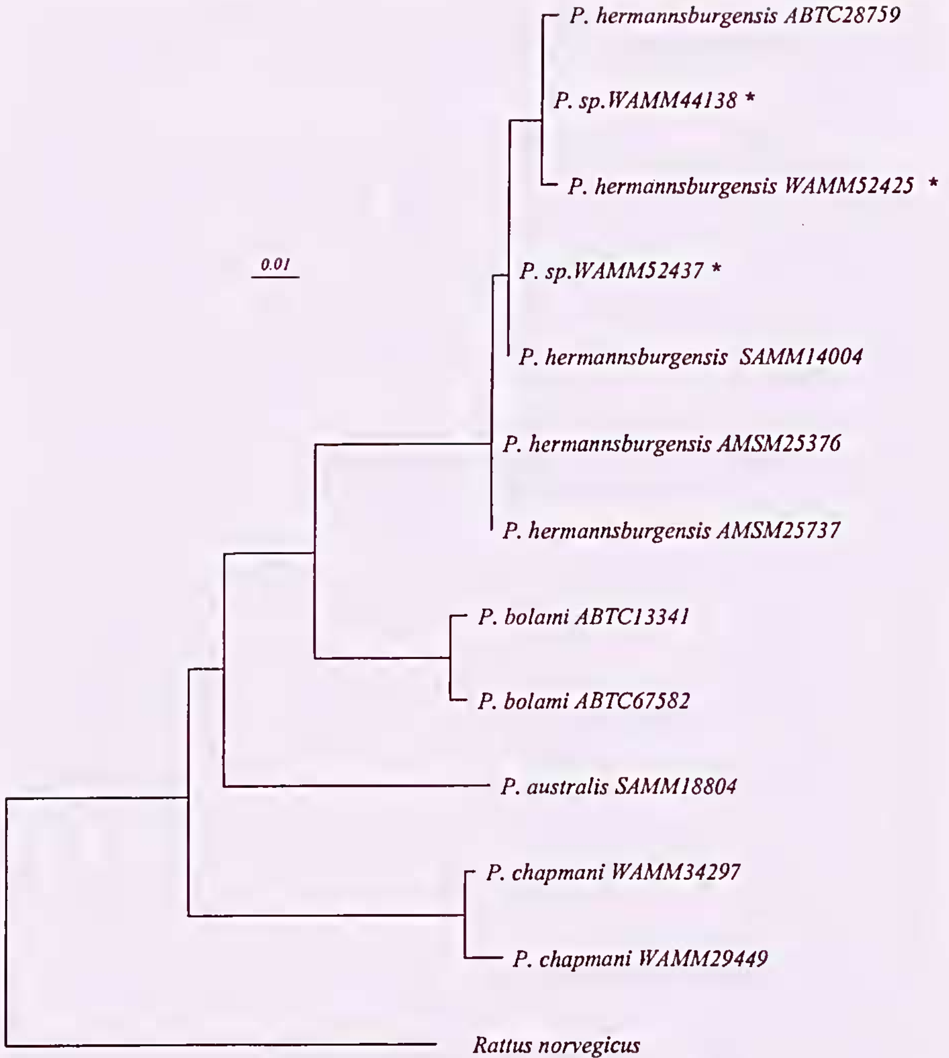

Figure 2. Neighbour-joining tree for the sequence data of Table 5, rooted using *Rattus norvegicus* as an outgroup. The sympatric *P.* sp. and *P. hermannsburgensis* animals are asterisked. The scale bar represents a Jukes-Cantor genetic distance of 0.01.

similar allozyme frequencies at all loci (maximum difference in allele frequency = 27% for *Idh-1*[a]), and none of these differences were statistically-significant at the p = 0.05 level. Thus the two forms are allozymically indistinguishable from one another.

mtDNA sequencing

Table 5 compares the mtDNA sequence data for the two

Table 4. A comparison of allozyme frequencies betwcen the two morphotypcs. Where an allozyme is not fixed, the percentage frequency of each allozyme is shown as a superscript. Total number of haploid genomes shown in brackets. All comparisons are non-significant based on calculating an exact probability for the appropriate M x N contingency table. (Raymond and Rousset 1995).

Locus	*Pseudomys* sp. (n=8)	*P. hermannsburgensis* (n=26)
Acyc	b	b^{88},c^{12}
Ada	a^{63},b^{37}	a^{85},b^{15}
Adh-2	b^{75},a^{25}	b^{96},a^{4}
Ap-1	b	b^{96},a^{4}
Ca-2	b^{87},a^{13}	b^{96},a^{4}
Dia	b	b^{88},a^{12}
Gda	b	b^{96},a^{4}
Got-2	b^{75},d^{25}	b^{77},d^{23}
Idh-1	a	$a^{73},c^{19},b^{4}.d^{4}$
Ldh	b^{87},a^{13}	b
Me	a	a^{96},b^{4}
6Pgd	b	b^{96},c^{4}
Pgm-1	a^{88},b^{12}	a
Pgm-2	c	c^{88},a^{8},d^{4}

Pseudomys sp. and one *P. hermannsburgensis* analyzed in this study against a number of reference sequences of *P. hermannsburgensis* plus three other congeneric species (*P. bolami*, *P. chapmani*, and *P. australis*). The three test sequences are all very similar, both to one another (only three variable sites) and to the reference specimens of *P. hermannsburgensis* (a maximum of four differences). In contrast, all seven Sandy Inland Mouse sequences differ from the *P. bolami* reference specimens at 20 or more sites (15 of which are fully-diagnostic between these two sibling species). The reference sequences for *P. chapmani* and *P. australis* are even more divergent.

Figure 2 presents a phylogram of the mtDNA sequences. As shown, the genetic affinities of all three Pilbara specimens clearly lie within *P. hermannsburgensis*, with the four described species all belonging to separate, well-defined lineages. This general pattern of within-species similarity and between-species distinctiveness has been consistent for all species of *Pseudomys* sequenced thus far for this portion of the *cytb gene* (Steve Donnellan, Evolutionary Biology Unit; unpublished).

DISCUSSION

The molecular genetic data reveal no evidence that the four specimens of the variant morphotype examined herein represent a species of *Pseudomys* distinct from *P. hermannsburgensis*. These specimens displayed molecular genetic profiles indistinguishable from those of other *P. hermannsburgensis*, both in sympatry and from several points across its geographic range. The inclusion of both allozyme and mtDNA datasets ensures that this is a very comprehensive assessment of genetic identity.

While this gracile form almost certainly does not represent a new biological species, our morphological analyses support

Table 5. Summary of the variable nucleotide positions in a 307 base-pair fragment of the mitochondrial *cyt-b* gene. Shown in bold are the three sequences generated in this study, compared to a selection of reference sequences taken from an existing Evolutionary Biology Unit database. (. = same base as for *P. australis* SAMM18804; ? = could not be determined)

Animal	Nucleotide Position		
	1	1111111111111111111	2222222222222222222222223
	11122233344555677890	0011223334456888999	0012223344555666778889990
	4703456803403068109440	3925173792810157167	5813690417067903814039581
P. australis SAMM18804	CCT?TCCATTTCCCCACTTTCA	CCCCCCCCACTGTTTGTTG	AATCTCTTACTTCACTTATCTTCTC
P. chapmani WAMM34297	TTCA.T.GC.C...T.TC....	.TTT..ATGTCACCCACCA	..A.CT..TTCAACT.CC...CA.A
P. chapmani WAMM29449	TTCA...GC.C...T.TC....	.TTT..ATGTCACCCAC.A	..A.CT..TTCAATT.CC...CA.A
P. bolami ABTC67582	..AC......CTT.T..CC...	.T..TTTT...ACC.A...	GGA.C.....CAAT.C.CCT.C.C.
P. bolami ABTC13341	..AC......CTT.T..CC...	.T..T.TT...ACC.A...	GGA.C..C..CAAT.C.CCT.C.C.
P. hermannsburgensis SAMM14004	..ACC.T....TTTT.TC.CTG	TT.TT.TT...AC..AC..	..AT......CAAC.C.CC.CC.C.
P. hermannsburgensis AMSM25377	..ACC....A.TTTTGTC.CTG	T..TT.TT...AC..AC..	..AT......CAAC.C.CC.CC.C.
P. hermannsburgensis AMSM25376	..ACC.T....TTTT.TC.CTG	.T.TT.TT...AC..AC..	..AT......CAAC.C.CC.CC.C.
P. hermannsburgensis ABTC28759	..ACC.T....TTTT.TC.CTG	.T.TT.TT...AC..AC..	..AT......CAAC.C.CC.CC.C.
***P. hermannsburgensis* WAMM52425**	**..ACC.T....TTTTGTC.CTG**	**T..TT.TT...AC..AC..**	**..AT..C...CAAC.C.CC.CC.C.**
***P. sp.*WAMM52437**	**..ACC.T....TTTT.TC.CTG**	**TT.TT.TT...AC..AC..**	**..AT......CAAC.C.CC.CC.C.**
***P. sp.*WAMM44138**	**..ACC.T....TTTTGTC.CTG**	**T..TT.TT...AC..AC..**	**..AT......CAAC.C.CC.CC.C.**

the notion that it is genuinely distinctive in external appearance when compared to typical *P. hermannsburgensis* from the Pilbara region and elsewhere. This distinctiveness is manifested in three of the four external characters examined, with gracile specimens being generally smaller, more slender-footed, and possessing relatively shorter ears. Despite no unequivocal indications that the skulls of these gracile animals were distinctive, there was a suggestion that subtle shape differences may become apparent if larger sample sizes had been available.

Within-species morphological variability features in most mammals. Such variability is most commonly encountered when comparing different populations, but nevertheless can and does occur within a population or region. Where such variability is marked and discontinuous, it may appear to mimic the presence of two or more morphologically-distinct species. This appears to be the case for *Pseudomys hermannsburgensis* in the Pilbara region of Western Australia. Elsewhere in Australia, other variant forms of what is presumably *P. hermannsburgensis* have been noted, involving both larger animals (*pers. comm.* Fred Ford) and animals which appear intermediate between *P. hermannsburgensis* and *P. bolami* (*pers. comm.* Cath Kemper). It remains to be seen whether these unusual animals are indeed simply morphotypic variants of the Sandy Inland Mouse, or whether some represent new taxa or hybrids with other species of *Pseudomys.* The present study highlights the advantages of using molecular genetic data to address the taxonomic identity of such animals and reinforces the need for voucher specimens and their tissues to be lodged with an appropriate museum.

We can only speculate as to the reasons why there are two discrete morphotypes of the Sandy Inland Mouse in the Pilbara. Perhaps these smaller, fully mature animals represent the consequences of a localized population response to the occasional good season by a small number of animals becoming reproductively mature at a much earlier stage and size. Alternatively, there may exist at low frequency one or more "partial-dwarfism" alleles among the various genes which influence growth in the *P. hermnannsburgensis* of that region. If these alleles are recessive and at low frequency in the population, then only a very small number of adults would display the "partially-dwarfed" phenotype. Such a situation occurs for example in laboratory rats (*Rattus norvegicus*), where mutant recessive alleles at four different genes are capable of producing fertile dwarf adults which range between 30-70% of normal adult body size (Hedrich 1990). Whatever the reason, it is clearly

important that taxonomists remain open to the reality that not all novel and distinctive morphological variants must automatically represent new species.

Of course the converse can occasionally be true, in that there are instances where different, morphologically-distinguishable species cannot unequivocally be diagnosed by molecular genetic analysis (Avise 1994; Richardson *et al.* 1986). However, such situations are generally limited to particular organismal groups which use visual cues for mate recognition (eg birds), to allopatric sister species, or to genetic analyses which are not comprehensive (i.e. only mtDNA data gathered, or only a small number of allozyme loci examined). As none of these three caveats applies to the present study, we are confident that the genetic diagnosis provided herein is valid. Nevertheless, such a conclusion can never deny the reality that genetically-determined differences in a small number of the genes which control morphology may be the only differences which characterize and therefore diagnose closely-related species. Thus, morphological data will always remain an essential component of any assessment of the taxonomic status of individuals and populations, both as a source of potential genetic markers of the speciation process, as well as the means by which taxa are formally described and subsequently diagnosed.

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